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<b>(21) International Application Number:</b> PCT/US83/00032 <b>(22) International Filing Date:</b> 11 January 1983 (11.01.83) <b>(31) Priority Application Numbers:</b> 339,826 414,053 <b>(32) Priority Dates:</b> 15 January 1982 (15.01.82) 2 September 1982 (02.09.82) <b>(33) Priority Country:</b> US <b>(71) Applicant:</b> CETUS CORPORATION [US/US]; 600 Bancroft Way, Berkeley, CA 94710 (US). <b>(72) Inventor:</b> INNIS, Michael, A. ; 3133 Carlson Street, Oakland, CA 94602 (US). <b>(74) Agents:</b> CIOTTI, Thomas, E.; Burns, Doane, Swecker & Mathis, Post Office Box 1404, Alexandria, VA 22313-1404 (US) et al.		<b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the          claims and to be republished in the event of the receipt          of amendments.</i>

**(54) Title:** INTERFERON-ALPHA 76

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1
Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile Cys
ATG GCC CTG TCC TTT TCT TTA CTG ATG GCC CTG CTG CTG CTC AGC TAC AAA TCC ATC TGT

21
Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu
TCT CTG GGC TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGC AGG GCC TTG ATA CTC

41
Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly
CTG GCA CAA ATG GGA AGA ATC TCT CAT TTC TCC TGC CTG AAG GAC AGA CAT CAT TTC GGA

61
Phe Pro Gln Gln Gln Phe Asp Gly His Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
TTC CCC GAG GAG GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC

81
His Gln Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp
CAT GAG ATG ATC CAG CAG ACC TTC AAT CTC TTC AOC ACA GAG GAC TCA TCT GCT GCT TGC

101
Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Gln
GAA CAG AGC CTC CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG CAA CTG AAT CAC CTG GAA

121
Ala Cys Val Ile Gln Gln Val Gly Val Gln Gln Thr Pro Leu Met Asn Gln Asp Ser Ile
GCA TGT CTG ATA CAG GAG GTT GGG CTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC

141
Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
CTG GCT CTG AGC AAA TAC TTC CAA AGA ATC ACT CTT TAT CTA ACA GAG AAG AAA TAC AOC

161
Pro Cys Ala Trp Gln Val Val Arg Ala Gln Ile Met Arg Ser Leu Ser Phe Ser Thr Asn
CCT TGT GCC TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA AAC

181
Leu Gln Lys Arg Leu Arg Arg Lys Asp
TTG CAA AAA AGA TTA AGG AGC AAG GAT

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**(57) Abstract**

New polypeptide, called IFN- $\alpha$ 76, produced by *E. coli* transformed with a newly isolated and characterized human IFN- $\alpha$  gene. The polypeptide exhibits interferon activities such as antiviral activity, cell growth regulation, and regulation of production of cell-produced substances.

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INTERFERON-ALPHA 76DescriptionTechnical Field

The invention is in the field of biotechnology. More particularly it relates to a polypeptide having interferon (IFN) activity, DNA that codes for the polypeptide, a recombinant vector that includes the DNA, a host organism transformed with the recombinant vector that produces the polypeptide, pharmaceutical compositions containing the polypeptide, and therapeutic methods employing the polypeptide.

Background Art

IFNs are proteins with antiviral, immunomodulatory, and antiproliferative activities produced by mammalian cells in response to a variety of inducers (see Stewart, W.E., The Interferon System, Springer-Verlag, New York, 1979). The activity of IFN is largely species specific (Colby, C., and Morgan, M. J., Ann. Rev. Microbiol. 25:333-360 (1971) and thus only human IFN can be used for human clinical studies. Human IFNs are classified into three groups,  $\alpha$ ,  $\beta$ , and  $\gamma$ , (Nature, 286:110, (1980)). The human IFN- $\alpha$  genes compose a multigene family sharing 85%-95% sequence homology (Goeddel, D. V., et al, Nature 290:20-27 (1981) Nagata, S., et al, J. Interferon Research 1:333-336 (1981)). Several of the IFN- $\alpha$  genes have been cloned and expressed in E.coli (Nagata, S., et



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al, Nature 284:316-320 (1980); Goeddel, D. V., et al, Nature 287:411-415 (1980); Yelverton, E., et al, Nucleic Acids Research, 9:731-741, (1981); Streuli, M., et al, Proc Nat Acad Sci (USA), 78:2848-2852. The  
 5 resulting polypeptides have been purified and tested for biological activities associated with partially purified native human IFNs and found to possess similar activities. Accordingly such polypeptides are potentially useful as antiviral, immunomodulatory, or  
 10 antiproliferative agents.

A principal object of the present invention is to provide a polypeptide having interferon activity that is produced by an organism transformed with a newly isolated and newly characterized IFN- $\alpha$  gene.  
 15 This polypeptide is sometimes referred to herein as IFN- $\alpha$ 76. Other objects of the invention are directed to providing the compositions and organisms that are used to produce this polypeptide and to therapeutic compositions and methods that use this polypeptide as  
 20 an active ingredient.

#### Disclosure of the Invention

One aspect of the invention is a polypeptide having interferon activity and comprising the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln  
 MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu  
 GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet  
 IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer  
 LeuLeuGluLysPhe SerThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysVal  
 IleGlnGluValGly ValGluGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal  
 25 ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla  
 TrpGluValValArg AlaGluIleMetArg SerLeuSerPheSer ThrAsnLeuGlnLys  
 ArgLeuArgArgLys Asp



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A second aspect of the invention is a DNA unit or fragment comprising a nucleotide sequence that encodes the above described polypeptide.

5 A third aspect of the invention is a cloning vehicle or vector that includes the above described DNA.

10 A fourth aspect of the invention is a host organism that is transformed with the above described cloning vehicle and that produces the above described polypeptide.

A fifth aspect of the invention is a process for producing the above described polypeptide comprising cultivating said transformed host organism and collecting the polypeptide from the resulting culture.

15 Another aspect of the invention is a pharmaceutical composition having interferon activity comprising an effective amount of the above described polypeptide admixed with a pharmaceutically acceptable carrier.

20 Still another aspect of the invention is a method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the above described polypeptide to the human.

## 25 Brief Description of the Drawings

Figure 1 is a partial restriction map which shows the two XhoII restriction sites that produce a homologous 260 base pair DNA fragment from the IFN- $\alpha$ 1 and IFN- $\alpha$ 2 structural genes. Data for this map are  
30 from Streuli, M., et al Science, 209:1343-1347 (1980).

Figure 2 depicts the sequencing strategy used to obtain the complete DNA sequence of the IFN- $\alpha$ 76 gene coding region. Bacteriophage mp7: $\alpha$ 76-1



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DNA served as the template for sequences obtained with primers A, H and F and bacteriophage mp7: $\alpha$ 76-2 DNA was the template for sequences obtained with primers E and G. The crosshatched area of the gene depicts the region that encodes the 23 amino acid signal polypeptide and the open box depicts the region that encodes the mature polypeptide. The scale, in base pairs, is numbered with 0 representing the ATG start codon of preinterferon. The arrows indicate the direction and extent of sequencing with each primer.

Figure 3 is the nucleotide sequence of the structural gene coding for IFN- $\alpha$ 76 including some of the flanking 5'- and 3'- noncoding regions of the gene. The region coding for preinterferon and the mature polypeptide begins with the ATG codon at position 75 and terminates with the TGA codon at position 642.

Figure 4 is a partial restriction map of the coding region of the IFN- $\alpha$ 76 gene. The crosshatching represents the region that encodes the 23 amino acid signal peptide and the open box represents the gene coding sequence for the mature polypeptide. The scale, in base pairs, is numbered with 0 representing the ATG start codon of preinterferon.

Figure 5 shows the amino acid sequence of the 23 amino acid signal polypeptide and the 166 amino acid mature IFN- $\alpha$ 76 coded for by the gene depicted in Figure 3. The 189 amino acid sequence is displayed above the corresponding nucleotide sequence. Amino acid 24, cysteine, is the first amino acid of the mature IFN- $\alpha$ 76 protein.

Figure 6 is the DNA sequence of the E. coli trp promoter and the gene of Figure 3 which was inserted between the EcoRI and HindIII sites of the



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plasmid pBR322. The amino acid sequence of Figure 5 is written above the corresponding DNA sequence and the location of the restriction sites used in the construction of the expression plasmid are indicated.

5 Figure 7 is a diagram of the expression plasmid, pGW19.

#### Modes for Carrying Out the Invention

In general terms IFN- $\alpha$ 76 was made by identifying and isolating the IFN- $\alpha$ 76 gene by screening a  
10 library of human genomic DNA with an appropriate IFN- $\alpha$  DNA probe, constructing a vector containing the IFN- $\alpha$ 76 gene, transforming microorganisms with the vector, cultivating transformants that express IFN- $\alpha$ 76 and collecting IFN- $\alpha$ 76 from the culture. A preferred  
15 embodiment of this procedure is described below.

#### DNA Probe Preparation

Total cytoplasmic RNA was extracted from human lymphoblastoid cells, Namalwa, which had been induced for IFN production by pretreatment with  
20 5-bromodeoxyuridine (Tovey, M.G., et al, Nature 267:455-457 (1977)) and Newcastle Disease Virus (NDV). The poly(A) (polyadenylic acid)-containing messenger RNA (mRNA) was isolated from total RNA by chromatography on oligo(dT)-cellulose (type 3 from  
25 Collaborative Research; Aviv, H., and Leder, P., Proc Natl Acad Sci (USA), 69:1408-1412, (1972)) and enriched for IFN mRNA by density gradient centrifugation on 5%-20% sucrose gradients. Fractions containing IFN mRNA were identified by translating the  
30 mRNA by microinjecting aliquots of each fraction into Xenopus oocytes and determining the IFN activity of the products of the translations according to a method



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described by Colman, A., and Morser, J., Cell, 17:517-526 (1979).

The Namalwa cell human IFN enriched mRNA was used to construct complementary DNA (cDNA) clones in E. coli by the G/C tailing method using the PstI site of the cloning vector pBR322 (Bolivar, F., et al, Gene, 2:95-113 (1977)). A population of transformants containing approximately 50,000 individual cDNA clones was grown in one liter of medium overnight and the total plasmid DNA was isolated.

The sequences of two IFN- $\alpha$  clones (IFN- $\alpha$ 1 and IFN- $\alpha$ 2) have been published (Streuli, M., et al, Science, 209:1343-1347 (1980)). Examination of the DNA sequences of these two clones revealed that the restriction enzyme XhoII would excise a 260 bp fragment from either the IFN- $\alpha$ 1 or the IFN- $\alpha$ 2 gene (see Figure 1). XhoII was prepared in accordance with the process described by Gingeras, T.R., and Roberts, R.J., J Mol Biol, 118:113-122 (1978).

One mg of the purified total plasmid DNA preparation was digested with XhoII and the DNA fragments were separated on a preparative 6% polyacrylamide gel. DNA from the region of the gel corresponding to 260 bp was recovered by electroelution and recloned by ligation into the BamHI site of the single strand bacteriophage ml3:mp7. Thirty-six clones were picked at random, the single stranded DNA isolated therefrom, and the DNA was sequenced. The DNA sequences of four of these clones were homologous to known IFN- $\alpha$  DNA sequences. Clone mp7: $\alpha$ -260, with a DNA sequence identical to IFN- $\alpha$ 1 DNA (Streuli, M. et al, Science, 209:1343-1347 (1980)) was chosen as a highly specific hybridization probe for identifying additional IFN- $\alpha$  DNA sequences. This clone is hereinafter referred to as the "260 probe."



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Screening of Genomic DNA Library

In order to isolate other IFN- $\alpha$  gene sequences, a  $^{32}\text{P}$ -labelled 260 probe was used to screen a library of human genomic DNA by in situ hybridization. The human gene bank, prepared by Lawn, R.M., et al, Cell, 15:1157-1174 (1978), was generated by partial cleavage of fetal human DNA with HaeIII and AluI and cloned into bacteriophage  $\lambda$  Charon 4A with synthetic EcoRI linkers. Approximately 800,000 clones were screened, of which about 160 hybridized with the 260 probe. Each of the 160 clones was further characterized by restriction enzyme mapping and comparison with the published restriction maps of 10 chromosomal IFN genes (Nagata, S., et al, J Interferon Research, 1:333-336 (1981)). One of the clones, hybrid phage  $\lambda 4A:\alpha 76$  containing a 15.5 kb insert, was characterized as follows. A DNA preparation of  $\lambda 4A:\alpha 76$  was cleaved with HindIII, BglII, and EcoRI respectively, the fragments separated on an agarose gel, transferred to a nitrocellulose filter (Southern, E.M., J Mol Biol, 98:503-517 (1977)) and hybridized with  $^{32}\text{P}$ -labelled 260 probe. This procedure localized the IFN- $\alpha 76$  gene to a 2.0 kb EcoRI restriction fragment which was then isolated and recloned, in both orientations, by ligation of the fragment into EcoRI cleaved ml3:mp7. The two subclones are designated mp7: $\alpha 76$ -1 and mp7: $\alpha 76$ -2. The -1 designation indicates that the single-stranded bacteriophage contains insert DNA complementary to the mRNA (the minus strand) and the -2 designation indicates that the insert DNA is the same sequence as the mRNA (the plus strand).



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### Sequencing of the IFN- $\alpha$ 76 Gene

The Sanger dideoxy-technique was used to determine the DNA sequence of the IFN- $\alpha$ 76 gene. The strategy employed is diagrammed in Figure 2, the DNA sequence thus obtained is given in Figure 3, and a partial restriction enzyme map of the IFN- $\alpha$ 76 gene is illustrated in Figure 4. Unlike many genes from eukaryotic organisms, but analogous to other IFN chromosomal genes which have been characterised, the DNA sequence of this gene demonstrates that it lacks introns. Homology to protein sequence information from these known IFN- $\alpha$  genes made it possible to determine the correct translational reading frame and thus allowed the entire 166 amino acid sequence of IFN- $\alpha$ 76 to be predicted from the DNA sequence as well as a precursor segment, or signal polypeptide, of 23 amino acids (Figure 5).

The DNA sequence of the IFN- $\alpha$ 76 gene and the amino acid sequence predicted therefrom differ substantially from the other known IFN- $\alpha$  DNA and IFN- $\alpha$  amino acid sequences. Nagata, S., et al, (J Interferon Research, 1:333-336, (1981)) describe isolating two IFN- $\alpha$  genes, IFN- $\alpha$ 4a and IFN- $\alpha$ 4b, that differ by five nucleotides which entails 2 amino acid changes in the proteins expressed thereby. The sequence of IFN- $\alpha$ b is given in European Patent Application No. 81300050.2. The IFN- $\alpha$ 76 structural gene differs from the IFN- $\alpha$ 4b gene by 5 nucleotides which entails 4 amino acid changes in the corresponding proteins: a single nucleotide change creates an amino acid substitution of alanine for threonine at amino acid number 14 of the mature protein; a double nucleotide change creates an amino acid substitution of alanine for glutamine at amino acid number 19 of



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the mature protein; a single nucleotide change creates an amino acid substitution of alanine for threonine at amino acid number 51 of the mature protein; and, a single nucleotide change creates an amino acid change of glutamate for valine at amino acid number 114 of the mature protein.

#### Plasmid Preparation and Host Transformation

Assembly of the plasmid for direct expression of the IFN- $\alpha$ 76 gene involved replacing the DNA fragment encoding the 23 amino acid signal polypeptide of preinterferon with a 120 bp EcoRI/Sau3A promoter fragment (E.coli trp promoter, operator, and trp leader ribosome binding site preceding an ATG initiation codon) and using the naturally occurring HindIII site, 142 bp 3'- of the TGA translational stop codon, to insert the gene into a vector derived from the plasmid pBR322. The complete DNA sequence of the promoter and gene fragments inserted between the EcoRI and HindIII sites of pBR322 is shown in Figure 6 which also shows the exact location of relevant cloning sites. Details of the construction are described below.

The coding region for mature IFN- $\alpha$ 76 encompasses a Sau3A site between codons for amino acids 2 and 3 and an AvaI site between codons for amino acids 39 and 40. The 111 bp Sau3A to AvaI fragment was isolated on a 6% polyacrylamide gel following a Sau3A/AvaI double-digest of the 2.0 kb EcoRI genomic fragment. Similarly, the 528 bp fragment from the AvaI site between codons for amino acids 39 and 40 and the HindIII site 142 nucleotides 3'- of the translational stop codon was isolated on a 5% polyacrylamide gel. These two fragments, together with a 120 bp



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EcoRI to Sau3A E.coli promoter fragment were ligated together in a four way directed ligation into the EcoRI to HindIII site of pBR322. The promoter fragment, which contains a synthetic HindIII restriction site, ATG initiation codon, the initial cysteine codon (TGT) common to all known IFN- $\alpha$ s, and Sau3A "sticky end", had been constructed previously. The ligation mixture was used to transform E.coli MM294 (Backman, K., et al, Proc Natl Acad Sci (USA) 73:4174-10 4178 (1976)). The desired correct transformant, one out of 24 screened, was identified by restriction enzyme mapping of colonies which hybridized to a  $^{32}\text{P}$ -labelled IFN- $\alpha$  genomic fragment. Figure 7 is a diagram of the final expression plasmid obtained, which 15 is designated pGW19. Other prokaryotic hosts such as bacteria other than E.coli may, of course, be transformed with this or other suitable constructs to replicate the IFN- $\alpha$ 76 gene and/or to produce IFN- $\alpha$ 76. IFN- $\alpha$ 76 produced in accordance with the 20 invention is believed to be distinct from the corresponding native protein in several respects. Firstly, because the IFN- $\alpha$ 76 gene was expressed by bacterial hosts that utilize N-formyl-methionine and/or methionine to initiate translation, some or all of the bacterially produced IFN- $\alpha$ 76 molecules are preceded by an 25 N-formyl-methionine or methionine group. Some of the N-formyl-methionine or methionine groups could be removed by natural in vivo bacterial cleavage mechanisms. This would result in a mixture of molecules, 30 some of which would include an initial N-formyl-methionine or methionine and others that would not. All such IFN- $\alpha$ 76 molecules, those containing an initial N-formyl-methionine or methionine, those not containing an N-formyl-methionine or methionine and



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any mixture thereof, are encompassed by the present invention. Secondly, the amino acid residues of the bacterially produced polypeptide are unsubstituted whereas the residues of the native protein may be substituted with sugar groups, ACTH or other moieties. Also, native IFN- $\alpha$  extracts consist of mixtures of various IFN molecules whereas the bacterially produced IFN- $\alpha$ 76 is homogeneous; that is, bacterially produced IFN- $\alpha$ 76 does not contain functionally related polypeptides. Accordingly, the invention contemplates producing IFN- $\alpha$ 76-containing compositions having biological activity that is attributable solely to IFN- $\alpha$ 76 and/or said terminal N-formyl-methionine or methionine derivatives thereof.

#### 15 Cultivation of Transformants

Bacteria transformed with the IFN- $\alpha$ 76 gene may be cultivated in an appropriate growth medium, such as a minimum essential medium, that satisfies the nutritional and other requirements needed to permit the bacteria to grow and produce IFN- $\alpha$ 76. If the bacteria are such that the protein is contained in their cytoplasm, the IFN- $\alpha$ 76 may be extracted from the cells by lysing the cells such as by sonication and/or treatment with a strong anionic solubilizing agent such as sodium dodecyl sulfate. Further purification of the extract may be achieved by affinity chromatography, electrophoresis, or other protein purification techniques.

#### 25 Biological Testing of IFN- $\alpha$ 76

IFN- $\alpha$ 76-containing cell sonicates were tested in vitro and found to have the following activities: (1) inhibition of viral replication of



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vesicular stomatitis virus (VSV) and herpes simplex virus-1 (HSV-1); (2) inhibition of tumor cell growth; (3) inhibition of colony formation by tumor cells in soft agar; (4) activation of natural killer (NK) cells; (5) enhancement of the level of 2',5'-oligoadenylate synthetase (2',5'-A); and (6) enhancement of the double-stranded RNA-dependent protein kinase. The sonicates were active in inhibiting viral infection in both human and other mammalian cells such as hamster, monkey, mouse, and rabbit cells.

The tests show that IFN- $\alpha$ 76 exhibits antiviral activity against DNA and RNA viruses, cell growth regulating activity, and an ability to regulate the production of intracellular enzymes and other cell-produced substances. Accordingly, it is expected IFN- $\alpha$ 76 may be used to treat viral infections with a potential for interferon therapy such as chronic hepatitis B infection, ocular, local, or systemic herpes virus infections, influenza and other respiratory tract virus infections, rabies and other viral zoonoses, arbovirus infections, and slow virus diseases such as Kuru and sclerosing panencephalitis. It may also be useful for treating viral infections in immunocompromised patients such as herpes zoster and varicella, cytomegalovirus, Epstein-Barr virus infection, herpes simplex infections, rubella, and progressive multifocal leukoencephalopathy. Its cell growth regulating activity makes it potentially useful for treating tumors and cancers such as osteogenic sarcoma, multiple myeloma, Hodgkin's disease, nodular, poorly differentiated lymphoma, acute lymphocytic leukemia, breast carcinoma, melanoma, and nasopharyngeal carcinoma. The fact that IFN- $\alpha$ 76 increases protein kinase and 2',5'-oligoadenylate synthetase



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indicates it may also increase synthesis of other enzymes or cell-produced substances commonly affected by IFNs such as histamine, hyaluronic acid, prostaglandin E, tRNA methylase, and aryl hydrocarbon hydrolase. Similarly, it may be useful to inhibit enzymes commonly inhibited by IFNs such as tyrosine amino transferase, glycerol-3-phosphate dehydrogenase glutamine synthetase, ornithine decarboxylase, S-adenosyl-1-methionine decarboxylase, and UDP-N-acetylglucosamine-dolichol monophosphate transferase. The ability of the IFN- $\alpha$ 76 to stimulate NK cell activity is indicative that it may also possess other activities such as the abilities to induce macrophage activity and antibody production and to effect cell surface alterations such as changes in plasma membrane density or cell surface charge, altered capacity to bind substances such as cholera toxin, concanavalin A and thyroid-stimulating hormone, and change in the exposure of surface gangliosides.

Pharmaceutical compositions that contain IFN- $\alpha$ 76 as an active ingredient will normally be formulated with an appropriate solid or liquid carrier depending upon the particular mode of administration being used. For instance, parenteral formulations are usually injectable fluids that use pharmaceutically and physiologically acceptable fluids such as physiological saline, balanced salt solutions, or the like as a vehicle. Oral formulations, on the other hand, may be solid, eg tablet or capsule, or liquid solutions or suspensions. IFN- $\alpha$ 76 will usually be formulated as a unit dosage form that contains in the range of  $10^4$  to  $10^7$  international units, more usually  $10^6$  to  $10^7$  international units, per dose.



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IFN- $\alpha$ 76 may be administered to humans in various manners such as orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, and subcutaneously. The particular mode of administration and dosage regimen will be selected by the attending physician taking into account the particulars of the patient, the disease and the disease state involved. For instance, viral infections are usually treated by daily or twice daily doses over a few days to a few weeks; whereas tumor or cancer treatment involves daily or multidaily doses over months or years. IFN- $\alpha$ 76 therapy may be combined with other treatments and may be combined with or used in association with other chemotherapeutic or chemopreventive agents for providing therapy against viral infections, neoplasms, or other conditions against which it is effective. For instance, in the case of herpes virus keratitis treatment, therapy with IFN has been supplemented by thermocautery, debridement and trifluorothymidine therapy.

Modifications of the above described modes for carrying out the invention, such as, without limitation, use of alternative vectors, alternative expression control systems in the vector, and alternative host microorganisms and other therapeutic or related uses of IFN- $\alpha$ 76, that are obvious to those of ordinary skill in the biotechnology, pharmaceutical, medical and/or related fields are intended to be within the scope of the following claims.



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Claims

1. A polypeptide having interferon activity and comprising the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln  
MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu  
GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet  
IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer  
LeuLeuGluLysPhe SerThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysVal  
IleGlnGluValGly ValGluGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal  
ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla  
TrpGluValValArg AlaGluIleMetArg SerLeuSerPheSer ThrAsnLeuGlnLys  
ArgLeuArgArgLys Asp.

5 2. The polypeptide of claim 1 wherein the polypeptide consists essentially of said amino acid sequence.

3. The polypeptide of claim 1 or 2 wherein the initial cysteine residue of the amino acid  
10 sequence is preceded by an N-formyl-methionine group.

4. The polypeptide of claim 1 or 2 wherein the amino acid residues of said sequence are unsubstituted.

5. IFN- $\alpha$ 76.

15 6. A composition having interferon activity and comprising a mixture of:

(a) a polypeptide having the amino acid sequence

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln  
MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu  
GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet  
IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer



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LeuLeuGluLysPhe SerThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysVal  
 IleGlnGluValGly ValGluGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal  
 ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla  
 TrpGluValValArg AlaGluIleMetArg SerLeuSerPheSer ThrAsnLeuGlnLys  
 ArgLeuArgArgLys Asp

and;

(b) a polypeptide having said amino acid sequence wherein the initial cysteine residue of the sequence is preceded by an N-formyl-methionine or methionine group.

7. The composition of claim 6 wherein the amino acid residues of said sequence are unsubstituted.

10 8. A composition having interferon activity comprising a polypeptide having the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln  
 MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu  
 GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet  
 IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer  
 LeuLeuGluLysPhe SerThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysVal  
 IleGlnGluValGly ValGluGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal  
 ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla  
 TrpGluValValArg AlaGluIleMetArg SerLeuSerPheSer ThrAsnLeuGlnLys  
 ArgLeuArgArgLys Asp

or a mixture of said polypeptide and a polypeptide  
 15 having said sequence wherein the initial cysteine residue is preceded by an N-formyl-methionine or methionine group wherein the interferon activity of the composition is attributable to said polypeptide or to said mixture.

20 9. A DNA unit consisting of a nucleotide sequence that encodes the polypeptide of claim 1 or 5.



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10. The DNA unit of claim 9 wherein the nucleotide sequence is:

TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG  
GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC TCT CAT  
TTC TCC TGC CTG AAG GAC AGA CAT GAT TTC GGA TTC CCC  
GAG GAG GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA  
GCC ATC TCT GTC CTC CAT GAG ATG ATC CAG CAG ACC TTC  
AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG GAA  
CAG AGC CTC CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG  
CAA CTG AAT GAC CTG GAA GCA TGT GTG ATA CAG GAG GTT  
GGG GTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC  
CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT CTT TAT  
CTA ACA GAG AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT  
GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA  
AAC TTG CAA AAA AGA TTA AGG AGG AAG GAT.

11. A cloning vehicle that includes the DNA unit of claim 9 or 10.

12. The cloning vehicle of claim 11 wherein the cloning vehicle is a plasmid.

13. The cloning vehicle of claim 11 wherein the cloning vehicle is the plasmid pGW19.

10 14. A host that is transformed with the cloning vehicle of claim 11 and produces IFN- $\alpha$ 76.

15. The host of claim 13 wherein the host is a prokaryote.



-18-

16. The host of claim 14 wherein the host organism is E.coli.

17. A host that is transformed with the cloning vehicle of claim 13 and produces IFN- $\alpha$ 76, 5 wherein the host is E.coli.

18. A process for producing IFN- $\alpha$ 76 comprising cultivating the host of claim 14 and collecting IFN- $\alpha$ 76 from the resulting culture.

19. A process of producing IFN- $\alpha$ 76 comprising 10 cultivating the host organism of claim 16 and collecting IFN- $\alpha$ 76 from the resulting culture.

20. A process for producing IFN- $\alpha$ 76 comprising cultivating the host organism of claim 17 and collecting IFN- $\alpha$ 76 from the resulting culture.

15 21. A pharmaceutical composition comprising an effective amount of the polypeptide of claim 1, 2 or 5 admixed with a pharmaceutically acceptable vehicle or carrier.

20 22. A pharmaceutical composition comprising an effective amount of the composition of claim 6 or 8 admixed with a pharmaceutically acceptable vehicle or carrier.

25 23. A method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the polypeptide of claim 1, 2 or 5 to said human.



-19-

24. A method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the composition of claim 6 or 8 to said human.

5 25. The method of claim 23 wherein the therapy is for treating a viral infection, providing cell growth regulation, or regulating the production of a cell-produced substance.

10 26. The method of claim 24 wherein the therapy is for treating a viral infection, providing cell growth regulation, or regulating the production of a cell-produced substance.

27. A method of providing antiviral therapy to a mammal comprising administering a viral infection  
15 inhibiting amount of the polypeptide of claim 1, 2 or 5 to the mammal.



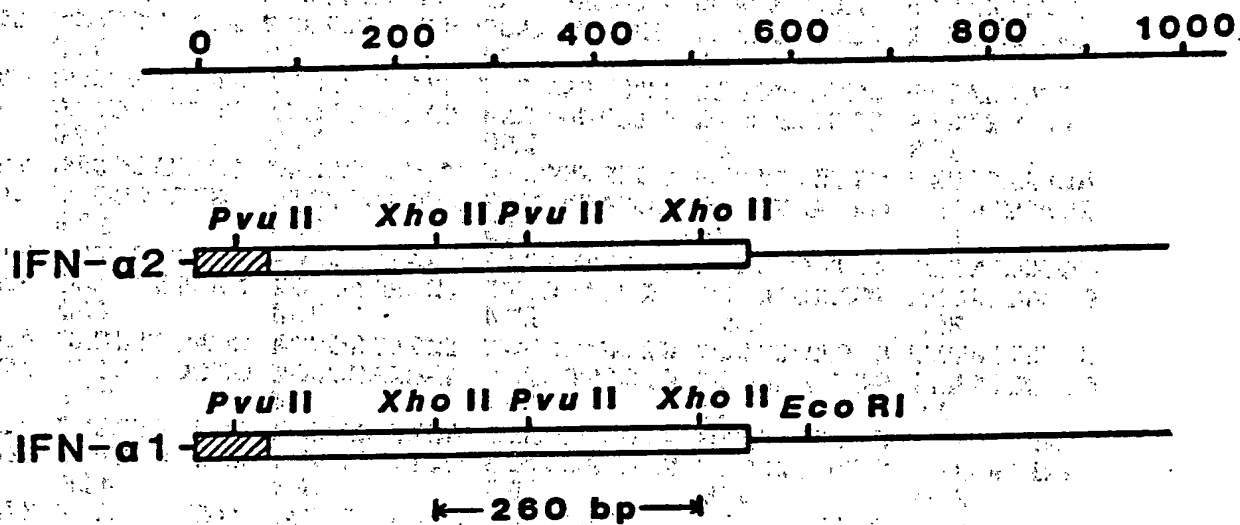


Figure 1

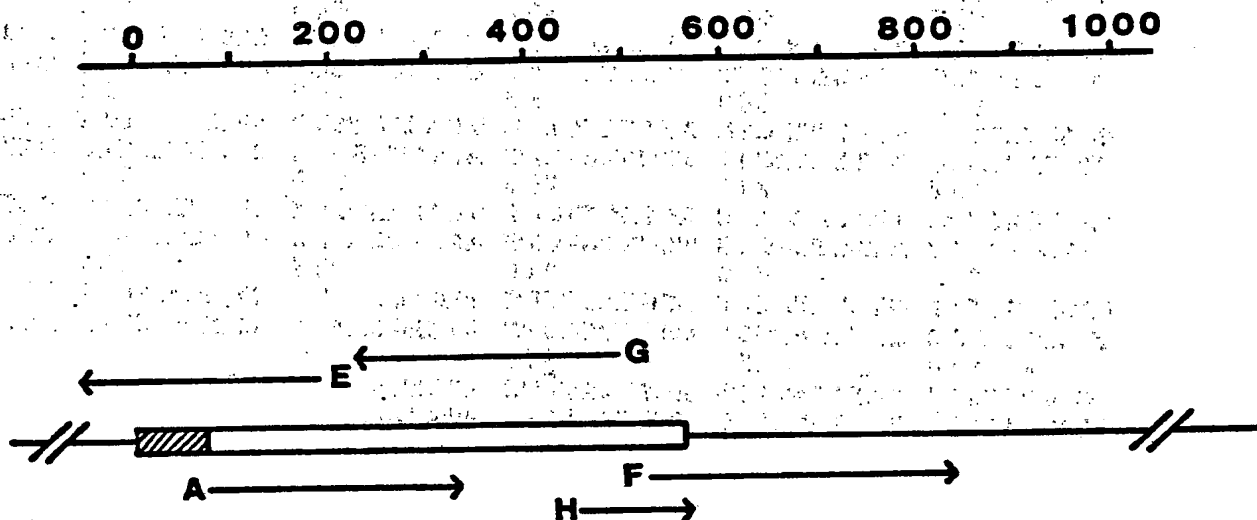


Figure 2

10	20	30	40	50	60
GTCTTCAGAA	AACCTAGAGG	CCGAAGTTCA	AGGTTATCCA	TCTCAAGTAG	CCTAGCAATA
CAGAAGTCTT	TTGGATCTCC	GGCTTCAAGT	TCCAATAGGT	AGAGTTCATC	GGATCGTTAT
70	80	90	100	110	120
TTTGCAACAT	CCCAATGGCC	CTGTCCTTTT	CTTTACTGAT	GGCCGTGCTG	GTGCTCAGCT
AAACGTTGTA	GGGTTACGGG	GACAGGAAAA	GAAATGACTA	CCGGCAGCAG	CACGAGTCGA
130	140	150	160	170	180
ACAAATCCAT	CTGTTCTCTG	GGCTGTGATC	TGCCTCAGAC	CCACAGCCTG	GGTAATAGGA
TGTTTAGGTA	GACAAGAGAC	CCGACACTAG	ACGGAGTCTG	GGTGTCCGAC	CCATTATCCT
190	200	210	220	230	240
GGGCCTTGAT	ACTCCTGGCA	CAAATGGGAA	GAATCTCTCA	TTTCTCCTGC	CTGAAGGACA
CCCGGAACTA	TGAGGACCGT	GTTTACCCTT	CTTAGAGAGT	AAAGAGGACG	GACTTCCTGT
250	260	270	280	290	300
GACATGATTT	CGGATTCCTC	GAGGAGGAGT	TTGATGGCCA	CCAGTTCAG	AAGGCTCAAG
CTGTACTAAA	GCCTAAGGGG	CTCCTCCTCA	AACCTACCGT	GGTCAAGGTC	TTCCGAGTTC
310	320	330	340	350	360
CCATCTCTGT	CCTCCATGAG	ATGATCCAGC	AGACCTTCAA	TCTCTTCAGC	ACAGAGGACT
GGTAGAGACA	GGAGGTACTC	TACTAGGTCT	TCTGGAAGTT	AGAGAAGTCG	TGTCTCCTGA
370	380	390	400	410	420
CATCTGCTGC	TTGGGAACAG	AGCCTCCTAG	AAAAATTTTC	CACTGAACCT	TACCAGCAAC
GTAGACGACG	AACCCTTGTC	TCGGAGGATC	TTTTTAAAG	GTGACTTGAA	ATGGTCGTTG
430	440	450	460	470	480
TGAATGACCT	GGAAGCATGT	GTGATACAGG	AGGTTGGGGT	GGAAGAGACT	CCCCTGATGA
ACTTACTGGA	CCTTCGTACA	CACTATGTCC	TCCAACCCCA	CCTTCTCTGA	GGGGACTACT
490	500	510	520	530	540
ATGAGGACTC	CATCCTGGCT	GTGAGGAAAT	ACTTCCAAAG	AATCACTCTT	TATCTAACAG
TACTCCTGAG	GTAGGACCGA	CACTCCTTTA	TGAAGGTTTC	TTAGTGAGAA	ATAGATTGTC
550	560	570	580	590	600
AGAAGAAATA	CAGCCCTTGT	GCCTGGGAGG	TTGTCAGAGC	AGAAATCATG	AGATCCCTCT
TCTTCTTTAT	GTCGGGAACA	CGGACCCTCC	AACAGTCTCG	TCTTTAGTAC	TCTAGGGAGA
610	620	630	640	650	660
CGTTTTCAAC	AAACTTGCAA	AAAAGATTAA	GGAGGAAGGA	TTGAAACCTG	GTTCAACATG
GCAAAAGTTG	TTTGAACGTT	TTTTCTAATT	CCTCCTTCCT	AACCTTGAC	CAAGTTGTAC
670	680	690	700	710	720
GAAATGATCC	TGATTGACTA	ATACATTATC	TCACACTTTC	ATGAGTTCTT	CCATTTCAAA
CTTTACTAGG	ACTAACTGAT	TATGTAATAG	AGTGTGAAAG	TACTCAAGAA	GGTAAAGTTT
730	740	750	760	770	780
GACTCACTTC	TATAACCACC	ACGAGTTGAA	TCAAAATTTT	CAAATGTTTT	CAGCAGTGTG
CTGAGTGAAG	ATATTGGTGG	TGCTCAACTT	AGTTTTAAAA	GTTTACAAAA	GTCGTACAC
790	800	810	820	830	840
AAGAAGCTTG	GTGTATACCT	GTGCAGGCAC	TAGTCCTTTA	CAGATGACAA	TGCTGATGTC
TTCTTCGAAC	CACATATGGA	CACGTCCGTG	ATCAGGAAAT	GTCTACTGTT	ACGACTACAG
850	860	870			
TCTGTTTCATC	TATTTATTTA	AATATTTATT	TATTTT		
AGACAAGTAG	ATAAATAAAT	TTATAAATAA	ATAAAA		

Figure 3



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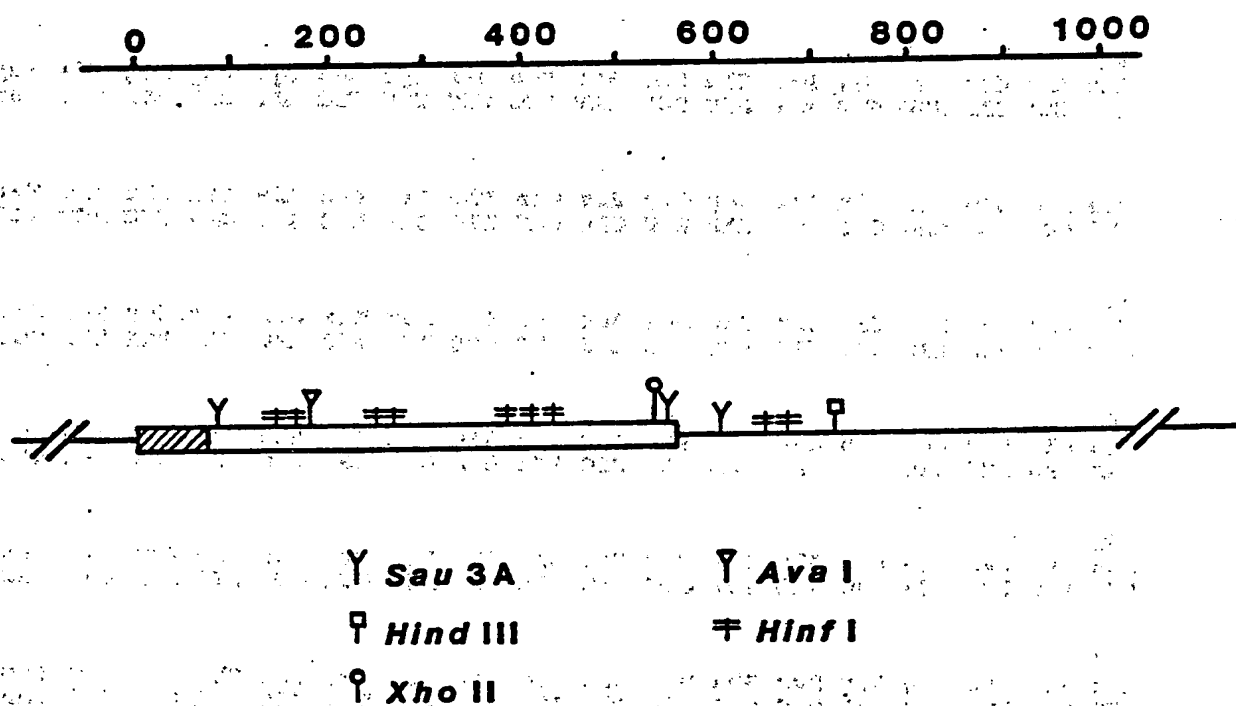


Figure 4

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1  
Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile Cys  
ATG GCC CTG TCC TTT TCT TTA CTG ATG GCC GTG CTG GTG CTC AGC TAC AAA TCC ATC TGT

21  
Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu  
TCT CTG GGC TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG GCC TTG ATA CTC

41  
Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly  
CTG GCA CAA ATG GGA AGA ATC TCT CAT TTC TCC TGC CTG AAG GAC AGA CAT GAT TTC GGA

61  
Phe Pro Glu Glu Glu Phe Asp Gly His Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu  
TTC CCC GAG GAG GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC

81  
His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp  
CAT GAG ATG ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG

101  
Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu  
GAA CAG AGC CTC CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG CAA CTG AAT GAC CTG GAA

121  
Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile  
GCA TGT GTG ATA CAG GAG GTT GGG GTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC

141  
Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser  
CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT CTT TAT CTA ACA GAG AAG AAA TAC AGC

161  
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn  
CCT TGT GCC TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA AAC

181  
Leu Gln Lys Arg Leu Arg Arg Lys Asp  
TTG CAA AAA AGA TTA AGG AGG AAG GAT

Figure 5



1  
GAA TTC CGA CAT CAT AAC GGT TCT GGC AAA TAT TCT GAA ATG AGC TGT TGA CAA TTA ATC  
 Eco RI

61  
 ATC GAA CTA GTT AAC TAG TAC GCA AGT TCA CGT AAA AAG GGT ATC GAT AAG CTT ATG TGT Met Cys

121  
 Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met  
GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG GCC TTG ATA CTC CTG GCA CAA ATG  
 Sau 3A

181  
 Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Glu Glu  
 GGA AGA ATC TCT CAT TTC TCC TGC CTG AAG GAC AGA CAT GAT TTC GGA TTC CCC GAG GAG  
 Ava I

241  
 Glu Phe Asp Gly His Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile  
 GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG ATC

301  
 Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser Leu  
 CAG CAG ACC TTC AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG GAA CAG AGC CTC

361  
 Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile  
 CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG CAA CTG AAT GAC CTG GAA GCA TGT GTG ATA

421  
 Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg  
 CAG GAG GTT GGG GTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC CTG GCT GTG AGG

481  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp  
 AAA TAC TTC CAA AGA ATC ACT CTT TAT CTA ACA GAG AAG AAA TAC AGC CCT TGT GCC TGG

541  
 Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys Arg  
 GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA AAC TTG CAA AAA AGA

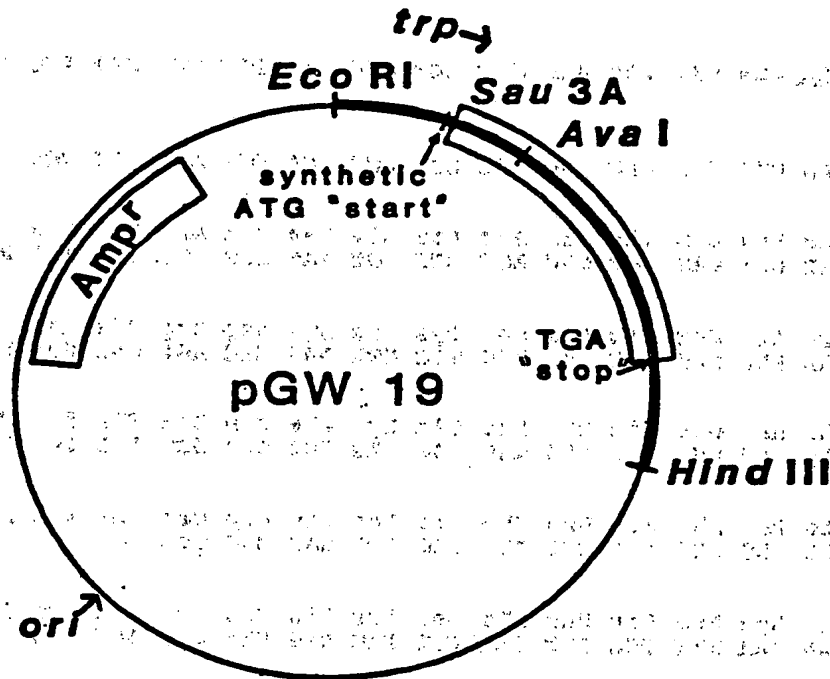
601  
 Leu Arg Arg Lys Asp \*\*\*  
 TTA AGG AGG AAG GAT TGA AAC CTG GTT CAA CAT GGA AAT GAT CCT GAT TGA CTA ATA CAT

661  
 TAT CTC ACA CTT TCA TGA GTT CTT CCA TTT CAA AGA CTC ACT TCT ATA ACC ACC ACG AGT

721  
 TGA ATC AAA ATT TTC AAA TGT TTT CAG CAG TGT GAA GAA GCT T  
 Hind III

Figure 6





## IFN- $\alpha$ 76 Expression Plasmid

Figure 7

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 83/00032

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>3</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>3</sup> : C 12 N 15/00; C 07 C 103/52; C 12 P 21/02; A 61 K 45/02; C 07 H 21/04; C 12 N 1/20 // C 12 R 1/19																	
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched <sup>4</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border: 1px solid black; padding: 5px;">Classification System</th> <th style="border: 1px solid black; padding: 5px;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">IPC<sup>3</sup></td> <td style="border: 1px solid black; padding: 10px; vertical-align: top;">C 07 C; C 12 N; A 61 K; C 12 R</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>5</sup></div>			Classification System	Classification Symbols	IPC <sup>3</sup>	C 07 C; C 12 N; A 61 K; C 12 R											
Classification System	Classification Symbols																
IPC <sup>3</sup>	C 07 C; C 12 N; A 61 K; C 12 R																
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category <sup>6</sup></th> <th style="width: 70%; padding: 5px;">Citation of Document, <sup>15</sup> with indication, where appropriate, of the relevant passages <sup>17</sup></th> <th style="width: 20%; padding: 5px;">Relevant to Claim No. <sup>18</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">Nature, volume 290, 5 March 1981, D.Goeddel et al.: "The structure of eight distinct cloned human leukocyte interferon C DNA's" pages 20-26, see the entire document --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,4,8-12</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">Nature, volume 287, 2 October 1980, G.Allen et al.: "A family of structural genes for human lymphoblastoid (leukocyte-type) interferon", pages 408-411, see the entire document --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,2,4</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">Science, volume 212, 5 June 1981, R.M.Lawn et al.: "DNA Sequence of two closely linked human leukocyte interferon genes", pages 1159-1162, see the entire document --</td> <td></td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">Nucleic Acids Research, volume 9, no. 3, 1981, E. Yelverton et al.: "Bacterial synthesis of a novel human leukocyte interferon", pages 731-741, see the entire document (cited in the application)</td> <td style="text-align: center; vertical-align: top; padding: 5px;">./.</td> </tr> </tbody> </table>			Category <sup>6</sup>	Citation of Document, <sup>15</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>	Y	Nature, volume 290, 5 March 1981, D.Goeddel et al.: "The structure of eight distinct cloned human leukocyte interferon C DNA's" pages 20-26, see the entire document --	1,4,8-12	Y	Nature, volume 287, 2 October 1980, G.Allen et al.: "A family of structural genes for human lymphoblastoid (leukocyte-type) interferon", pages 408-411, see the entire document --	1,2,4	Y	Science, volume 212, 5 June 1981, R.M.Lawn et al.: "DNA Sequence of two closely linked human leukocyte interferon genes", pages 1159-1162, see the entire document --		Y	Nucleic Acids Research, volume 9, no. 3, 1981, E. Yelverton et al.: "Bacterial synthesis of a novel human leukocyte interferon", pages 731-741, see the entire document (cited in the application)	./.
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>•</sup> Special categories of cited documents: <sup>19</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Δ" document member of the same patent family</p> </div> </div>																	
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;">           Date of the Actual Completion of the International Search <sup>8</sup>  <div style="text-align: center; font-weight: bold; font-size: 1.2em;">19th April 1983</div> </td> <td style="width: 50%; border: 1px solid black; padding: 5px;">           Date of Mailing of this International Search Report <sup>9</sup>  <div style="text-align: center; font-weight: bold; font-size: 1.2em;">11 MAY 1983</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">           International Searching Authority <sup>1</sup>  <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div> </td> <td style="border: 1px solid black; padding: 5px;">           Signature of Authorized Officer <sup>10</sup>  <div style="text-align: right;">               G.L.M. Kruydenberg           </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <sup>8</sup> <div style="text-align: center; font-weight: bold; font-size: 1.2em;">19th April 1983</div>	Date of Mailing of this International Search Report <sup>9</sup> <div style="text-align: center; font-weight: bold; font-size: 1.2em;">11 MAY 1983</div>	International Searching Authority <sup>1</sup> <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <sup>10</sup> <div style="text-align: right;">               G.L.M. Kruydenberg           </div>											
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	EP, A, 0042246 (Cancer Institute of Japanese Foundation for Cancer Research) 23 December 1981, see claims 1-8	1,2,4,8-12
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V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>10</sup>

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers \_\_\_\_\_ because they relate to subject matter <sup>12</sup> not required to be searched by this Authority, namely:

oo) 23-27 (PCT Rule 39.1.iv)

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out <sup>13</sup>, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>11</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

1 / 6

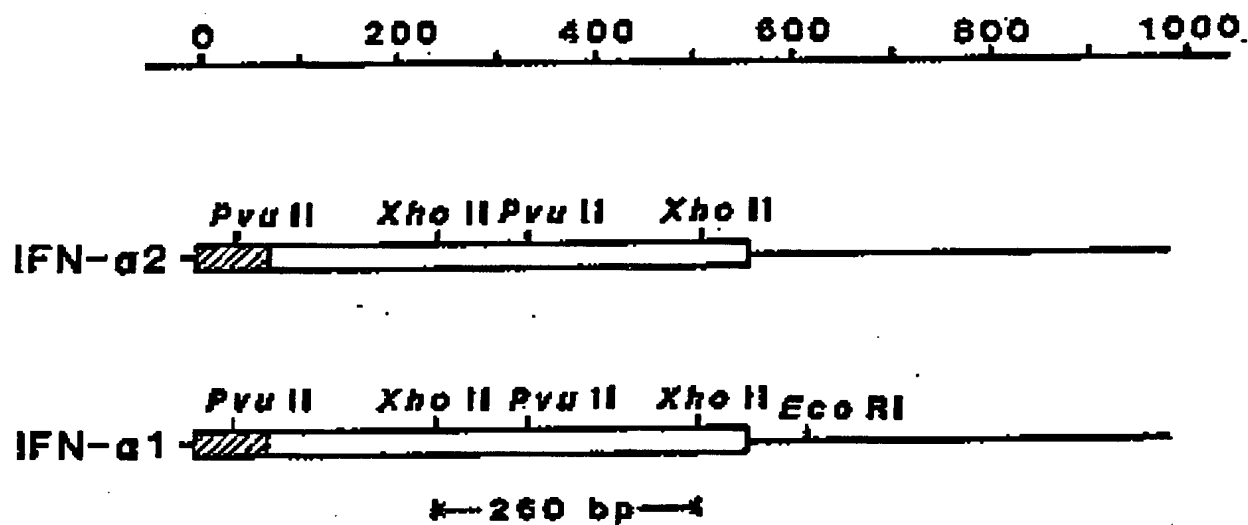


Figure 1

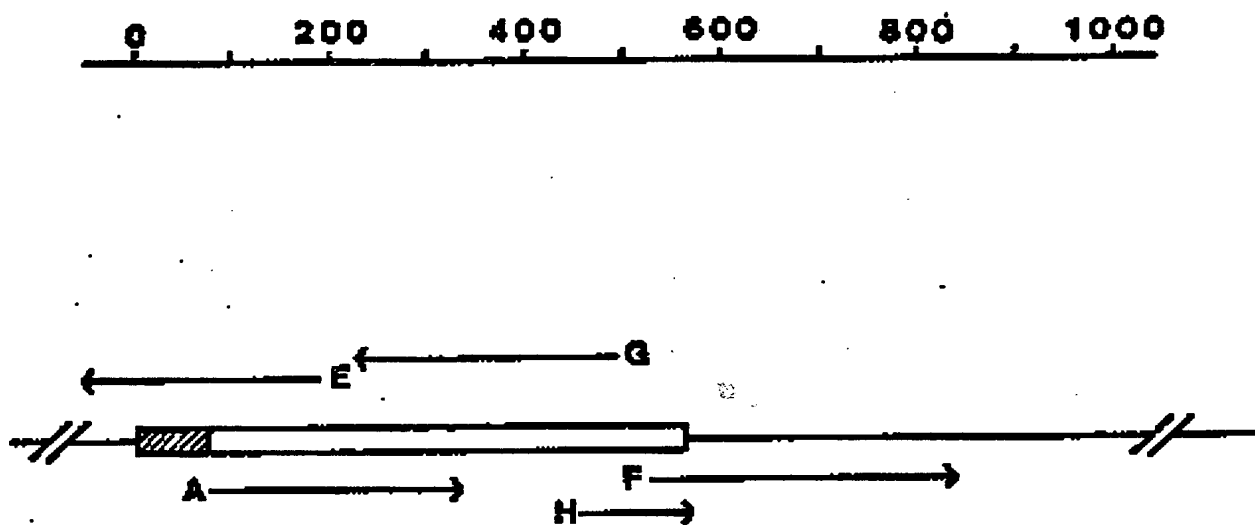


Figure 2

10	20	30	40	50	60
GTCTTCAAAA	AACCTAGAGG	CCGAAGTTCA	AGOTTATCCA	TCTCAAGTAG	CCTAGCAATA
CAGAAGTCTT	TTGGATCTCC	GGCTTCAAGT	TCCAATAGGT	AGAGTTCATC	GGATCGTTAT
70	80	90	100	110	120
TTTGCAACAT	CCCAATGGCC	CTGTCCTTTT	CTTACTGAT	GGCCGTGCTG	GTGCTCAGCT
AAACGTTGTA	GGGTTACCGG	GACAGGAAAA	GAAATGACTA	CCGGCAGGAC	CACGAGTCGA
130	140	150	160	170	180
ACAAATCCAT	CTGTTCTCTG	GGCTGTGATC	TGCCTCAGAC	CCACAGCCTG	GGTAATAGGA
TGTTTAGGTA	GACAAGAGAC	CCGACACTAG	ACGGAGTCTG	GGTCTCGGAC	CCATTATCCT
190	200	210	220	230	240
GGGCCTTGAT	ACTCCTGGCA	CAAATGGGAA	GAATCTCTCA	TTTCTCCTGC	CTGAAGGACA
CCCGGAACATA	TGAGGACCGT	GTTTACCCCT	CTTAGAGAGT	AAAGAGGACC	GACTTCCTGT
250	260	270	280	290	300
GACATGATTT	CGGATTCCCC	GAGGAGGAGT	TTGATGGCCA	CCAGTTCACG	AAGGCTCAAG
CTGTACTAAA	GCCTAAGGGG	CTCCTCCTCA	AACTACCGGT	GGTCAAGGTC	TTCCGAGTTC
310	320	330	340	350	360
CCATCTCTGT	CCTCCATGAG	ATGATCCAGC	ACACCTTCAA	TCTCTTCAGC	ACAGAGGACT
GGTAGAGACA	GGAGGTACTC	TACTAGGTCG	TCTGGAAGTT	AGAGAAGTCG	TGTCTCCTGA
370	380	390	400	410	420
CATCTGCTGC	TTGGGAACAG	AGCCTCCTAG	AAAAATTTTC	CACTGAACCT	TACCAGCAAC
GTAGACGACG	AACCCTTGTC	TCCGAGGATC	TTTTTAAAG	GTGACTTGAA	ATGCTCGTTG
430	440	450	460	470	480
TGAATGACCT	GGAAGCATGT	GTGATACAGG	AGGTTGGGGT	GGAAGAGACT	CCCCTGATGA
ACTTACTGGA	CCTTCGTACA	CACTATGTCC	TCCAACCCCA	CCTTCTCTGA	GGGGACTACT
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TACTCCTGAG	GTAGGACCGA	CACTCCTTTA	TGAAGGTTTC	TTAGTGAGAA	ATAGATTGTC
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TCTTCTTTAT	GTCGGGAACA	CGGACCCTCC	AACAGTCTCG	TCTTTAGTAC	TCTAGGGAGA
610	620	630	640	650	660
CGTTTTCAAC	AAACTTGCAA	AAAAGATTAA	GGAGGAAGGA	TTGAAACCTG	GTTCAACATG
GCAAAAGTTG	TTTGAACGTT	TTTTCTAATT	CCTCCTTCCT	AACCTTGGAC	CAAGTTGTAC
670	680	690	700	710	720
GAAATGATCC	TGATTGACTA	ATACATTATC	TCACACTTTC	ATGAGTTCTT	CCATTTCAA
CTTTACTAGG	ACTAAGTGAT	TATGTAATAG	AGTGTGAAAG	TACTCAAGAA	GGTAAAGTTT
730	740	750	760	770	780
GACTCACTTC	TATAACCACC	ACGAGTTGAA	TCAAAATTTT	CAAATGTTTT	CAGCAGTGTC
CTGAGTGAAG	ATATTGGTGG	TGCTCAACTT	AGTTTTAAAA	GTTTACAAAA	GTCGTACACAC
790	800	810	820	830	840
AAGAAGCTTG	GTGTATACCT	GTGCAGGCAC	TAGTCCTTTA	CAGATGACAA	TGCTGATGTC
TTCTTCGAAT	CACATATGGA	CACGTCCGTG	ATCAGGAAAT	GTCTACTGTT	ACGACTACAG
850	860	870			
TCTGTTCAAT	TATTTATTTA	AAATTTTATT	TATTTT		
AGACAAGTAG	ATAAATAAAT	TTATAAATAA	ATAAAA		

Figure 3

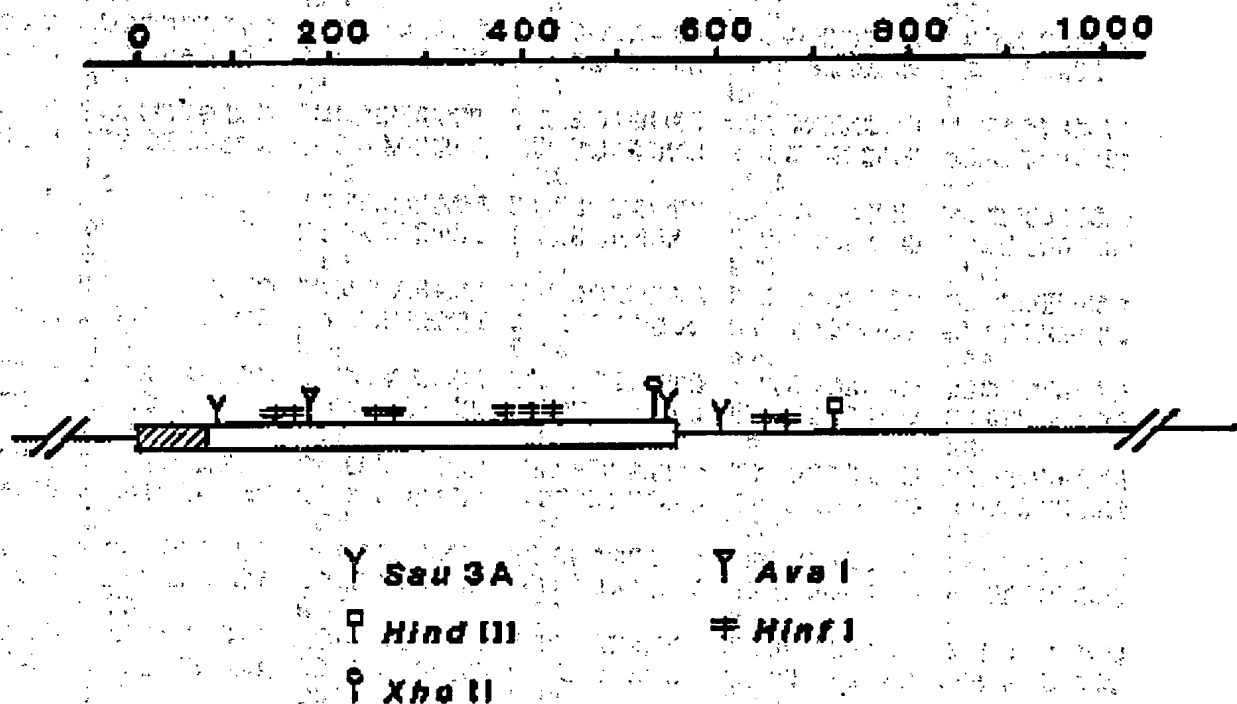


Figure 4

1 Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile Cys  
ATG GCC CTG TCC TTT TCT TTA CTG ATG GCC GTG CTG GTG CTC AGC TAC AAA TCC ATC TGT

21 Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu  
TCT CTG GGC TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG GCC TTG ATA CTC

41 Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly  
CTG GCA CAA ATG GGA AGA ATC TCT CAT TTC TCC TGC CTG AAG GAC AGA CAT GAT TTC GGA

61 Phe Pro Gln Glu Glu Phe Asp Gly His Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu  
TTC CCC GAG GAG GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC

81 His Gln Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp  
CAT GAG ATG ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG

101 Gln Gln Ser Leu Leu Gln Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu  
GAA CAG AGC CTC CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG CAA CTG AAT GAC CTG GAA

121 Ala Cys Val Ile Gln Gln Val Gly Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile  
GCA TGT GTG ATA CAG GAG GTT GGG GTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC

141 Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Gln Lys Lys Tyr Ser  
CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT CTT TAT CTA ACA GAG AAG AAA TAC AGC

161 Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn  
CCT TGT GCG TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA AAC

181 Leu Gln Lys Arg Leu Arg Arg Lys Asp  
TTG CAA AAA AGA TTA AGG AGG AAG GAT

Figure 5

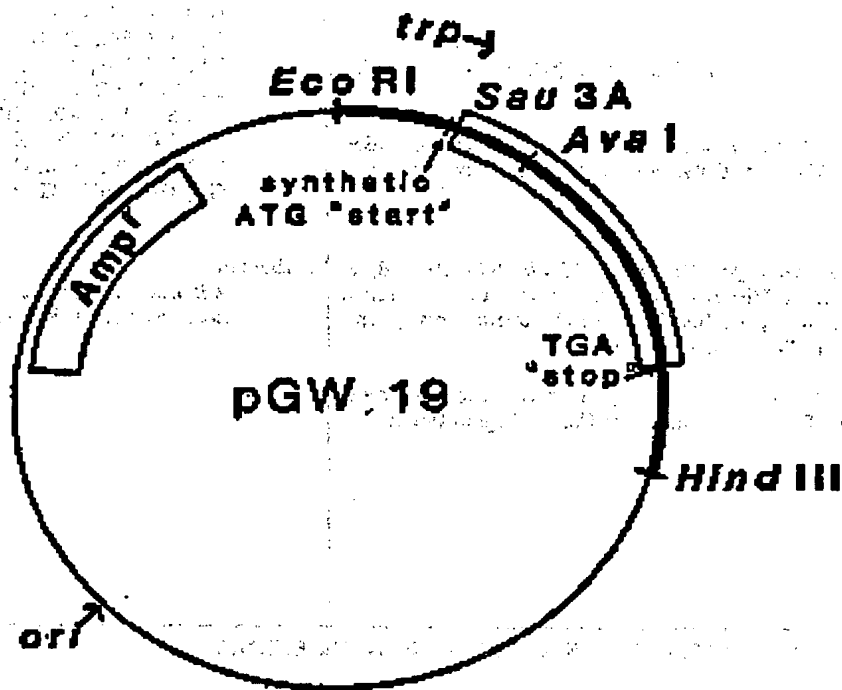


5/6

1  
 GAA TTC CGA CAT CAT AAC GGT TCT GGC AAA TAT TCT GAA ATG AGC TGT TGA CAA TTA ATC  
 Eco RI  
 61 Met Cys  
 ATC GAA CTA GTT AAC TAG TAC GCA AGT TCA CGT AAA AAG GGT ATC GAT AAG CTT ATG TGT  
 121  
 Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met  
 GAT CTT CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG GCC TTG ATA CTC CTG GCA CAA ATG  
 Sma 3A  
 181  
 Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Glu Glu  
 GGA AGA ATC TCT CAT TTC TCC TGC CTG AAG GAC AGA CAT GAT TTC GGA TTC GCG GAG GAG  
 Ase I  
 241  
 Glu Phe Asp Gly His Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile  
 GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG ATC  
 301  
 Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser Leu  
 CAG CAG ACC TTC AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG CAA CAG AGC CTC  
 361  
 Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile  
 CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG CAA CTG AAT GAC CTG GAA GCA TGT GTG ATA  
 421  
 Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg  
 CAG GAG GTT GGG GTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TCC ATC CTG GCT GTG AGG  
 481  
 Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp  
 AAA TAC TTC CAA AGA ATC ACT CTT TAT CTA ACA GAG AAG AAA TAC AGC CCT TGT GCC TGG  
 541  
 Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys Arg  
 GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC CTC TGG TTT TCA ACA AAC TTG CAA AAA AGA  
 601  
 Leu Arg Arg Lys Asp \*\*\*  
 TTA AGG AGG AAG GAT TGA AAC CTG GTT CAA CAT GGA AAT GAT CCT GAT TGA CTA ATA CAT  
 661  
 TAT CTC ACA CTT TCA TGA GTT CTT CCA TTT CAA AGA CTC ACT TCT ATA ACC ACC ACC AGT  
 721  
 TGA ATC AAA ATT TTC AAA TGT TTT CAG CAG TGT GAA GAA GGT T  
 Hind III

Figure 6





## IFN-α76 Expression Plasmid

Figure 7